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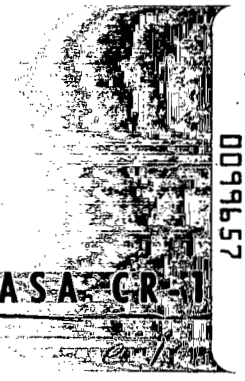

NASA CONTRACTOR REPORT

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STUDIES FOR STERILIZATION OF SPACE PROBE COMPONENTS

by Martin G. Koesterer

Prepared under Contract No. NASw-879 *by*
WILMOT CASTLE COMPANY
Rochester, N. Y.
for

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION • WASHINGTON, D. C. • MARCH 1965

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I SUMMARY

This final report for NASA contract NASw-879 constitutes a summary of the pertinent data obtained in our investigations of the effects of dry heat on microbial spores under a variety of conditions. These studies were undertaken to provide data in support of the development of sterilization systems to be utilized in the treatment of space-probe components. These studies have been limited to a temperature range between 80°C to 160°C with particular emphasis on temperatures of 125°C to 135°C respectively.

Studies were conducted to determine the level of natural contamination on various objects exposed to relatively dirty industrial production environments and the effect of dry heat treatment on these contaminated materials. Furthermore, a detailed and intensive study was conducted with several types of "off-the-shelf" electronic components to determine the presence and estimated concentrations of viable organisms entrapped within them.

The conclusions reached from an analysis of the data collected in these studies may be summarized as follows:

1. Dry heat treatment at 135°C for 24 hours has consistently killed large concentrations of

relatively resistant microbial spores in or on most carriers or substrates tested to date. It should be noted, however, that similar treatment was not effective in sterilizing native dried soil samples with their indigenous microbial flora.

2. The natural surface contamination of objects exposed to airborne microbial spore fallout, even in relatively dirty environments, did not attain significantly high concentration levels. All of these items could be sterilized at 135°C or less within just a few hours.

3. Despite the technical difficulties to the limitations inherent in a study of microbial contamination of electrical components it was shown that, with the extensive controls included in our study, relatively valid, though not absolute conclusions, could be drawn from the resulting data. These data indicate that only a small percentage of the components tested contained viable microorganisms entrapped within them. Furthermore, the estimates of levels of contamination in these components appear to be of a low order. It should be emphasized that items shown to be free of contamination may only reflect

the limitation of our test methods rather than a state of absolute sterility.

4. Preliminary investigations of dry heat treatment on bacterial spore populations under gaseous environments, in both closed and open systems, indicate that dry bacterial spores are not significantly more resistant in an inert gas environment, i.e. nitrogen, than they are in an atmosphere of dry air when heated to a temperature of 125°C.

5. The thermal death time data and curves for the relatively heat resistant microbial spore preparations are presented over a temperature range of 80°C to 160°C. These data should prove useful in the calculation and selection of optimum time-temperature cycles which may be effectively employed in the sterilization of individual components, space probes and, perhaps, entire space craft.

II INTRODUCTION

The research results presented in this final report for NASA Contract NASw-879 (FY 64-65) constitute an extension of the studies undertaken in support of the development of sterilization methods for space probe components by the Wilmot Castle Company Laboratories initiated under preceding contracts.[1] Emphasis has been placed on the investigation of dry heat as a sterilization agent in the temperature range of 80°C to 160°C, particularly at temperatures of 125 and 135°C.

A review of the pertinent literature has been presented in the final reports on the aforementioned previous contracts and no significant publications pertaining to this topic have come to our attention during the past year.

In the studies undertaken, attempts have been made to further define the various factors that may influence the effectiveness of dry heat as a sterilizing agent specifically aligned to the process as it may be applied to spacecraft components.

The areas of investigation undertaken or continued under this contract include:

A. Determination of the resistance of known micro-

1 NASA Contracts NASr-31 (FY 62-63) and NASw-550 (FY 63-64).

organisms on various carriers to dry heat.

1) Summary of results to-date on microorganisms to air at temperatures of 80° through 160°C.

2) dry heat treatment in heated nitrogen versus air, both circulating and non-circulating.

3) dry heat treatment during which the come-up time to the desired lethal treatment temperature is lengthened.

B. Determination of the thermal resistance of micro-organisms collected from natural environments to dry heat:

1) from the atmosphere on membrane filters, stainless steel strips, petri dishes, glass microscope slides, glass jars, and in liquid impingers followed by concentration on membrane filters.

2) on or in dirty objects and naturally contaminated materials.

3) encapsulated in solid materials.

C. Evaluation of microbial contamination of selected electronic components.

It is expected that thermal resistance data as presented previously, would be used to calculate times to sterilize at any one specific temperature (minimizing lags in heating)

(Schmidt, 1957) or that data as presented herein as thermal death time values could be mathematically integrated with heat penetration data so as to develop a general method for defining specific sterilization treatments (ie. lethality curves, which would take into account any killing during time to come to a specific temperature, as well as killing during time at that specific temperature or the total integrated lethality at temperatures above the lethal threshold). (Ball & Olson, 1957)

III RESEARCH INVESTIGATIONS

A. Determination of the resistance of known microorganisms on various carriers to dry heat.

1) Resistance of dry microbial spores to dry heat at temperatures of 80°C through 160°C.

The data in Figure 1 summarizes the results of our studies on the resistance (as thermal death times) of spores of several bacterial species from various sources on diverse carriers or substrates. This data is a compilation of experimental results obtained under the previous contracts, supplemented with additional experimental results obtained during this contract. The experimental techniques employed are those a) of partial survival i.e. determination of D values (Stumbo, 1948) from which F values (Schmidt, 1957) or times to sterilize could be calculated and b) sterility end-point determinations (thermal death times). The curves in Figure 1 are based on the values obtained by the latter technique. Results in terms of those conventionally employed for the former technique have been reported previously. (Koesterer 1962, 1963).

Specifically, it has been found of some 13 soils that have been screened to date that the indigenous microbial flora in FG soil required the longest times at all temperatures to sterilize. Spores of Bacillus coagulans and B.

subtilis var. niger are among the more resistant of the 20 known organisms that we have studied, although several unidentified resistant organisms isolated from heat treated soil samples have been shown to be as resistant as the known sporeformers. One isolate (541) has been shown to be more resistant than the known organisms, irrespective of carrier. The effect of carrier or substrate has been indicated previously but is again evident from the data in Figure 1 for spores of B. subtilis var. niger on paper strips, embedded in solid material or added to soil. The difference in thermal death times obtained for 0.1g FG soil samples when tryptone glucose yeast extract broth (TGYE) and thioglycollate broth (THIO) are employed as the sterility test media may also be noted in Figure 1, which emphasized the influence of recovery media on the results.

From the degree of linearity of these thermal death time curves, it appears that the time to sterilize any given preparation follows a predictable pattern over the noted temperature range. The similarity in the slopes of these curves suggests that the sterilization of these microbial preparations must depend on the same basic reaction in the spores, but that the availability of the reactive site varies with the preparation.

III A

2) Determination of the resistance of known microorganisms to dry heat treatment employing heated gaseous atmospheres of nitrogen or air, both circulating and non-circulating.

It has been intimated that an atmosphere of nitrogen might be employed around electronic components or in or around the entire spacecraft. All of our research to date on the resistance of microorganisms to dry heat has been performed in an atmosphere of air. It was considered that on the basis of thermal conductivities nitrogen should vary only slightly from air, in its effect on killing bacterial spores at any one temperature. It was decided to determine the times required to sterilize some of our spore preparations under nitrogen compared to those times established in an atmosphere of heated air. It can also be predicted that a heated circulating gas should impart more thermal energy per unit time than a non-circulating atmosphere, hence it can be reasoned that it should require less time to sterilize equivalent microbial preparations, other factors being equal.

A comparison was made of the efficacy of dry heat killing with both nitrogen and air at 125°C. Experiments quantitating the number of spores surviving at half-hour intervals and also determining the times required to sterilize

were performed on dry spores of Bacillus coagulans on paper strips sealed in ampoules with each respective gas. These ampoules were treated in a closely temperature regulated oil bath. The results are presented in Figure 2 and Table 1. The indication that nitrogen has a slightly lower lethality rate than air at the same temperature corroborates that of Phiel (1962). The observed experimental thermal death times in Table 1 agree closely with the calculated F values and the extrapolated sterility intercepts of the survivor curves in Figure 2.

Samples of the same spore preparations were also treated by placing the paper strips in the ends of several U-tubes connected together and flowing each of the heated gases over the strips in separate experimental runs.

The results of the various treatments are presented in Tables 2 and 3 for spores of B. subtilis var. niger and B. coagulans, respectively. These preliminary results indicate that the flowing heated gases and gases under a slight pressure (when air was evacuated and the ampoule equilibrated to atmospheric pressure with nitrogen and then the closed system heated) killed many times more organisms than when the spores were exposed to the gas of choice for a similar period with no forced circulation and equilibration at atmospheric pressure.

3) dry heat treatment during which the come-up time to the desired lethal treatment temperature is lengthened.

In all of our heat resistance work, reported previously and herein, the experiments were performed in such a manner so as to minimize any lag time required for the spores on any carrier to come to the desired treatment temperature. Most of the lag times have been very small (less than 5%) in relation to the overall treatment times required to sterilize all of the preparations.

However, it should be stated that in the application of dry heat to individual electronic components or to an entire spacecraft, the time to come to temperature will, in all probability, not be of such short duration. It may be desired that certain items be brought to the treatment temperature, and cooled at a controlled rate to avoid such effects as misalignment. Another factor which might lengthen the time to heat an item to the desired temperature is the actual heat transfer situations that could exist in shielded parts or components. If heat is applied in this manner ie. with other than a minimal lag factor, this fact must be taken into account and the procedure or treatment corrected.

In an attempt, to determine if and how longer lag times might affect the overall time to sterilize dry spores, some preliminary investigations were undertaken. The times required to kill dry spores of B. subtilis var. niger and B. coagulans on paper strips and also embedded in solid compounds when such preparations were heated from ambient to 135°C in an aluminum block unit equilibrated at ambient temperature were compared to those determined for other samples heated in the conventional manner by being placed in an aluminum block already equilibrated at 135°C.

Survivor curves for both treatments are presented in Figures 3 and 4 for B. subtilis var. niger and B. coagulans respectively. An additional 30-40 min was required (Curve B) to sterilize the preparations which underwent longer lag times over those samples which were brought to temperature more quickly. (Curve A) The actual killing at temperatures below the lethal threshold temperature appears to have been negligible when compared to the numbers of organisms in these preparations which were killed once the temperature was 100°C or greater. This phenomenon was also observed with samples of the same organisms embedded in the material referred to as Inlay Investment B, when treated in the same manner.

At present, the heat treatment recommended for planetary spacecraft does not take into account any degree of killing

of microorganisms which would occur at temperatures above the lethal threshold temperature but below 135°C. It is conceivable that programming a heat treatment equivalent to 24 hrs at 135°C might reduce the deleterious effects of heat on components or on an entire spacecraft. It may be more practical to take this phenomenon into account and to develop lethality curves specifically adapted to the thermal considerations of individual components or the composite thermal considerations that would exist in an entire spacecraft.

III B. Determination of thermal resistance of microorganisms collected from natural environments:

1) collected from the atmosphere:

It has been recommended that a more realistic sterilization cycle could be promulgated if the thermal resistance of the actual microbial contaminants, as they might occur naturally on a spacecraft were known. It should be pointed out, however, that this would not necessarily result in a reduction of the presently recommended thermal cycle but might elaborate more precisely the degree of reliability of that heat treatment.

Studies were undertaken to determine the level of microbial contamination that might accumulate naturally by fallout from the air on surfaces and to evaluate the time-temperature cycles of dry heat that would be required to sterilize items contaminated in this manner.

Other research groups are currently investigating the numbers and types of contaminating microorganisms which exist in actual clean room and spacecraft assembly areas. (Hoffman 1964, Vesley 1964)

In our studies samples of organisms were collected by entrapping the organisms from the air on

a) membrane filters

b) glass petri plates, microscope slides, and jars and

c) in liquid impingers, then concentrating the organisms on membrane filters followed by drying.

Microorganisms were collected on membrane filters, (Millipore type HA)[1] from various areas within the company's buildings and the results obtained are summarized in Table 4. Air was drawn through the filters for the times indicated, the filters were cut in half; one-half was assayed for the number of organisms and the other half placed in a sterile test tube and treated in dry heat in either the cylindrical aluminum block units, or in a hot air oven with forced circulation for various times at temperatures in the range of 115°-135°C. The heat treated filter halves were then assayed for sterility by adding sterile tryptone glucose yeast extract broth and incubated for no less than two weeks at 32°C. After that time, all tubes not showing evidence of growth were subcultured by streaking onto plate count agar slants or examined microscopically to confirm the presence or absence of organisms.

The results obtained (Table 5) indicated that very low levels of viable organisms were collected on the filters and that very brief cycles were adequate to sterilize them.

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1 Millipore Filter Corporation, Bedford, Massachusetts, (pore size 0.45 u I 0.2 u)

Additional studies employing impactor sieve samplers[1] and impinger samplers indicated that much higher levels of viable organisms were present in the same environmental atmosphere. Therefore, additional studies were initiated in which organisms were collected by allowing them to settle out on glass petri plates, microscope slides and into glass jars, as well as on stainless steel strips.

From the observation summarized in Table 5 it was found that all of these techniques resulted in the collection of similar levels of organisms per sample while impactors and impingers regularly indicated higher levels. The results of the heat treatments applied to the samples with their acquired microbial flora shows that all such samples were sterilized in five hours or less at 115°C, and in 1 hour or less at 120°C.

1 Technical Development Laboratories, Communicable Disease Center, U.S. Public Health Service, P.O. Box 769, Savannah, Georgia.

2) on dirty objects and naturally contaminated materials; and on artificially inoculated materials.

In an attempt to determine how resistant the actual types and levels of natural microbial contamination might be, compared to some of the aforementioned preparations of organisms of known high resistance, we determined the times required to sterilize the following:

a - dirty hardware items, objects which had accumulated natural contamination in an industrial manufacturing area.

The objects chosen included greasy or oily screws, nuts and bolts, metal scraps, washers, nails, pieces of tubing or conduit, etc. which had been lying around on the floor for undetermined times. Representative items were assayed and the frequency and range of the natural microbial contaminants estimated (Table 6). Most items had a very low bacterial population. The results are given for 17 items in Table 7.

Another set of items was treated in a forced hot air oven for various times at 135°C and then assayed for sterility in tryptone glucose extract broth

employing conventional aseptic transfer procedures. Microbial growth was evidenced in only a few culture vessels, including those containing items treated for times as short as 1 3/4 hours. Results of these tests are presented in Table 7.

After the incubation period was completed, approximately 100 spores of Bacillus subtilis var. niger were added to all culture vessels showing no evidence of microbial growth, to ascertain whether or not organisms might be inhibited by the presence of the item in the culture broth or that the medium was capable of supporting growth of organisms, if present. Several items did prevent the test inoculum from producing turbidity in the culture medium.

b - carbon black

Reports had indicated that organisms in the presence of carbon black were very difficult to kill, in fact, one report claimed that this material could not be sterilized. Small samples of various brands of activated carbon were assayed, heat treated, and the sterilized samples were re-inoculated in order to obtain some indication as to whether or not organisms in such materials could be killed. The

results are presented in Table 8 and indicate that carbon black, either with its natural contamination or with spores added to it, in any of several manners, can be sterilized with dry heat in times less than those required for soil samples (see Figure 1) at the temperature indicated.

III B

3) spores encapsulated in solid materials

A preliminary examination of the chemical and physical conditions that might be present during dry heat sterilization of electronic components indicated that vacuum, inert gases, and entrapment of spores within solids could have definite effects on sterilization. The results reported previously (Koesterer 1962) were obtained at only one temperature. Our experimental studies have been expanded to include other temperatures and another test organism. The results of the thermal death studies on spores of B. subtilis var. niger and B. coagulans added to various solid materials are given in Tables 9 and 10, respectively. The thermal death times for spores of B. subtilis var. niger entrapped in bridge model material are plotted and presented in Figure 1.

An analysis of Tables 9 and 10 by carriers or substrates shows that the spores of both organisms entrapped in various solids require much longer kill times than do same organisms on paper strips at all temperatures examined. TDT plots of the

data in these tables produce straight lines which approximate the slopes of the other TDT curves in Figure 1. All values fall short of those for the mesophilic population of FG soil as determined in thioglycollate broth, but are greater than those obtained for all three pure cultures on paper strips. Since the lag in heat penetration is approximately the same for all of these solid materials the variation in D values indicates that the ingredients of some of the solids may somehow effect the spores during heat treatment.

III C. Evaluation of microbial contamination of selected electronic components.

In order to reliably establish the level, as well as the absence of contaminating microorganisms in such items as electronic components, all work must be performed in an uncontaminated atmosphere with many controls being employed to ascertain that such an atmosphere is sterile.

The usual means of obtaining an uncontaminated atmosphere is to place a barrier around the objects to be evaluated and to sterilize the interior of that barrier and maintain sterility in the enclosed atmosphere. The conventional means of accomplishing this is to employ airtight plastic chambers such as are used in the germ-free animal program. The techniques for the use of such isolator systems have been reported by other groups as regards the testing or evaluation of microbial contamination inside electronic components (Phillips 1960, Cordaro 1962). Both of these groups have indicated that several of the electronic components examined harbored viable organisms. In their studies several components were tested at one time and a minimum of controls were employed. The fact remains that if contamination is found in one of a series of items tested, and in others tested simultaneously, then no unquestionable

result can be concluded as regards the presence or absence of contamination of the latter items. In order for the results to be meaningful, each item or component must be evaluated individually and with the necessary multiple controls.

Small flexible film isolator systems were adapted and modified for this task. The necessary equipment and two of the same type of components were placed in the isolator and the system closed. Ethylene oxide in a concentration of approximately 500 mg/liter of air space was admitted to sterilize the exterior of all the items and the atmosphere within the chamber. It was determined that 4-6 hrs was an adequate sterilizing exposure period, however, the ethylene oxide was usually allowed to remain in the isolators overnight. Air, sterilized by filtration through a cotton filter, was passed through the chamber for several hours on the following morning to remove all ethylene oxide gas from the isolator.

Two of each type of electronic component being tested were placed in the isolator along with additional sealed bottles and tubes of sterile bacteriological culture media. After the ethylene oxide treatment, one component was placed, whole, in a sterile medium blank.

This served as a control to indicate whether the exterior surface had indeed been sterilized by the ethylene oxide treatment.

Other control tests performed on each individual isolator (ie. for each individual component) included:

1. Sterility test on isolator
    a) sterilization indicators - seven spore strips in glassine envelopes were placed at various sites on the plastic isolator prior to the admission of gaseous ethylene oxide as follows:
        1) one on the external side of the exhaust filter which was taped shut on the exterior side during sterilization.
        2) four - one on interior of top, bottom, front and rear walls of isolator.
        3) two - one in a finger of each glove.

    These strips were aseptically transferred to blanks of culture media in the closed sterilized chamber after aeration.
    b) swab sterility test on various interior surfaces of the plastic isolator and
    c) contact tests for sterility of glove fingers - by dipping fingers in bottle containing

sterile culture broth were performed both prior to and after testing the component of interest for internal contamination.

2. Sterility tests on incoming filtered air
   a) by opening tube or bottle of sterile medium during aeration cycle.
   b) by continually bubbling the air through a midget liquid impinger containing sterile broth during the testing phase.
3. Sterility tests on instruments or tools employed were performed by dipping or rinsing and swabbing them in sterile media both prior to and after use.

The other component was then broken into pieces of the smallest size possible and these were placed in a blank of trypticase soy broth. A one milliliter aliquot of this blank was removed and added to a second blank. After all operations in the isolator were completed all culture vessels were sealed, removed and incubated for two weeks at 32°C. The culture vessels were examined periodically for turbidity, which might indicate bacterial growth. The culture vessels were opened and aliquots of the medium were streaked on tryptone glucose agar slants and incubated for two additional weeks at 32°C as a further check for bacterial growth. A sample showing no evidence of the presence or growth of

microorganisms, in any of these tests was considered to possess no internal contamination within the limitations employed in these tests.

Following the completed incubation period for the above tests and controls, all culture tubes not showing evidence of bacterial growth were inoculated with approximately 100 spores of B. subtilis var. niger. These inoculated vessels, after incubation were subcultured onto TGYE agar slants to determine whether the medium was capable of supporting microbial growth. Growth in this step indicated that the electronic component or the materials of the component would not inhibit growth of spores if they were present.

A summary of the results obtained on the components tested is presented in Table 11. With the controls employed it can be reliably assumed that all components were not internally sterile and where contamination was evidenced, the level of such contaminants was low. It should not be assumed, due to the incomplete reduction of the component to particles smaller than those capable of harboring microorganisms, that those components not presenting evidence of contamination are truly sterile.

An itemized account of the individual test on each component is given in Table 12. A close examination of

this table indicates that there were failures in sterilization of the isolator systems, breaks in sterility, and/or possibilities of transfer of contamination within the isolator test system. Thus positive conclusions as to the presence of contamination within some individual components cannot be reliably made. The fact that such conditions did occur points out the value of having performed adequate controls in the conduct of this study.

Considering the inherent pitfalls and difficulties encountered in this study, it is dubious that additional effort along these lines will yield any more definitive data than those qualitative observations indicated by the results of this study.

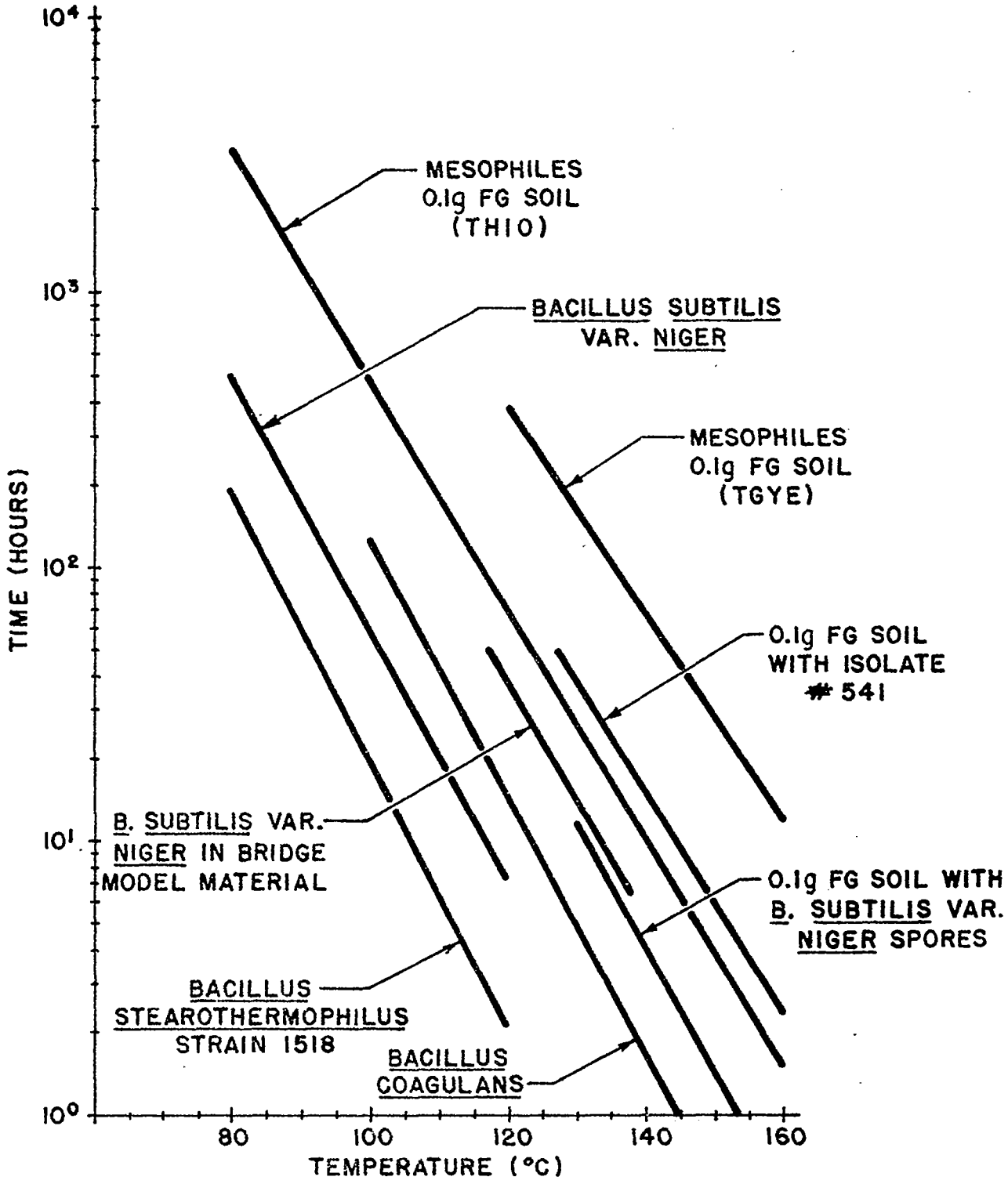

FIGURE 1. RESISTANCE OF DRY SPORES FROM THREE BACTERIAL SPECIES AND THE BACTERIAL SPORE POPULATION OF GARDEN SOIL TO DESTRUCTION BY DRY HEAT

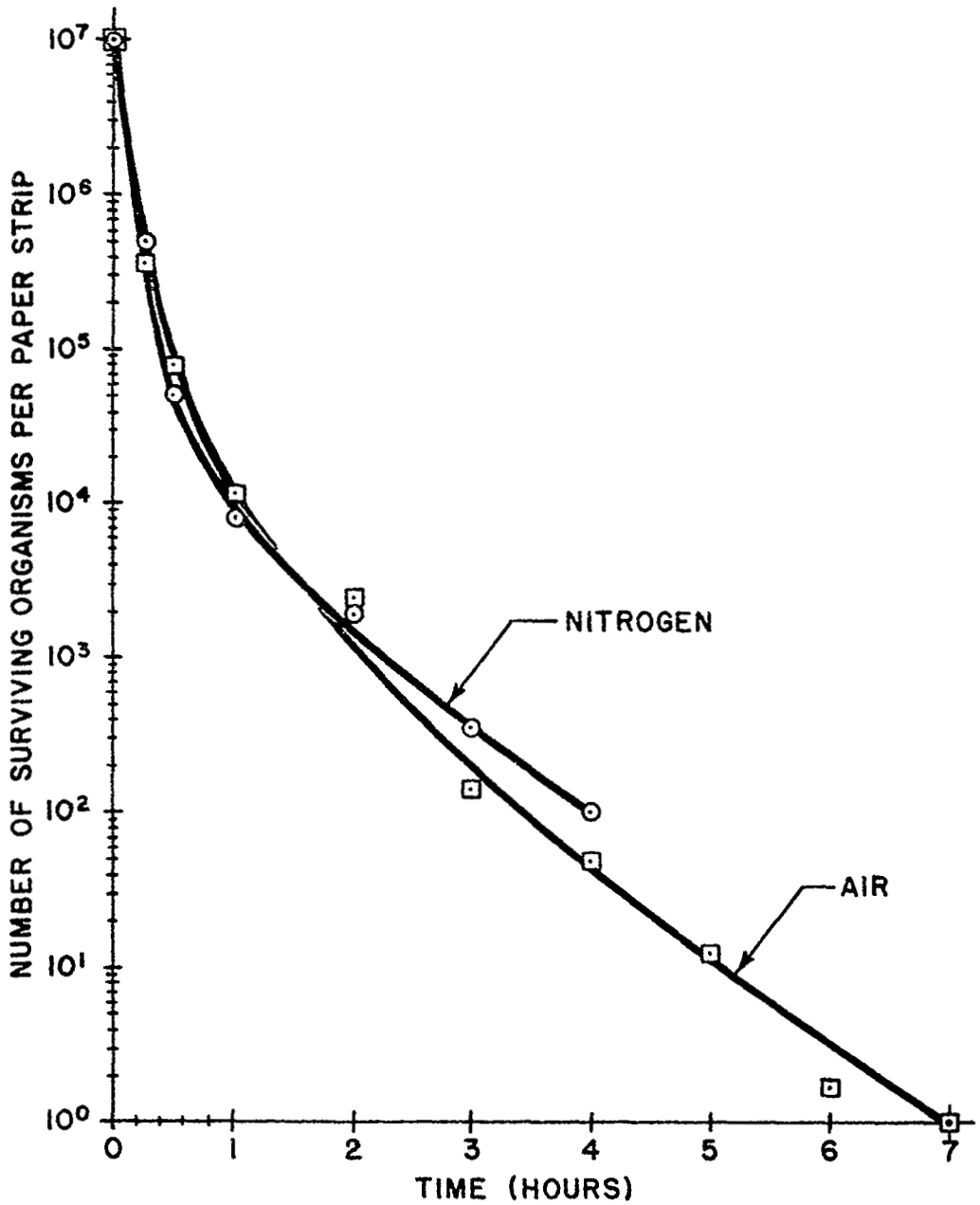

FIGURE 2. EFFECTS OF DRY HEAT TREATMENT AT 125°C. ON DRY SPORES OF BACILLUS COAGULANS ON PAPER STRIPS IN AN ATMOSPHERE OF NITROGEN COMPARED TO AIR

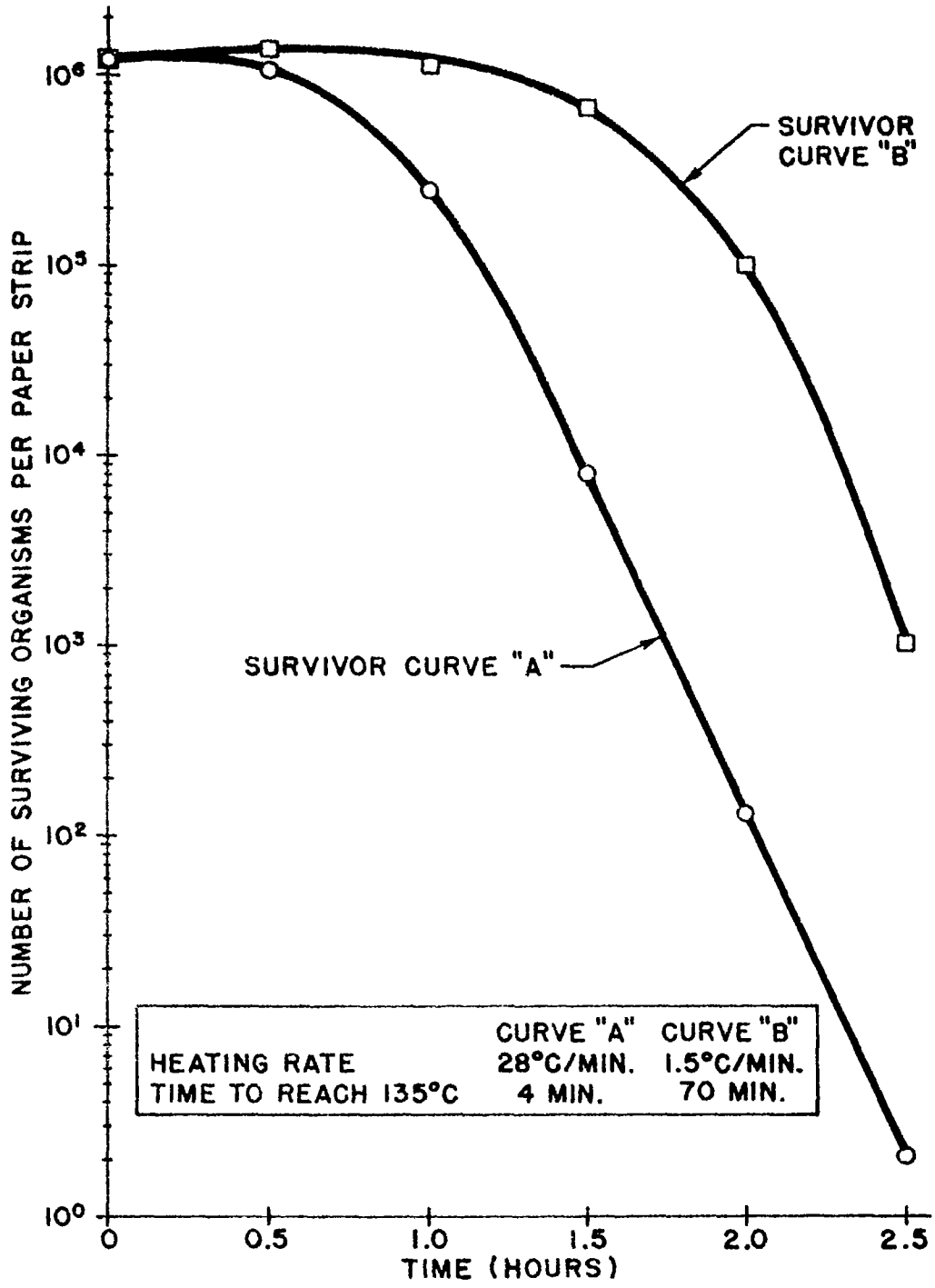

FIGURE 3. EFFECT OF THE WARM-UP RATE TO 135°C. ON DRY SPORES OF BACILLUS SUBTILIS VAR. NIGER ON PAPER STRIPS WHEN EXPOSED TO DRY HEAT

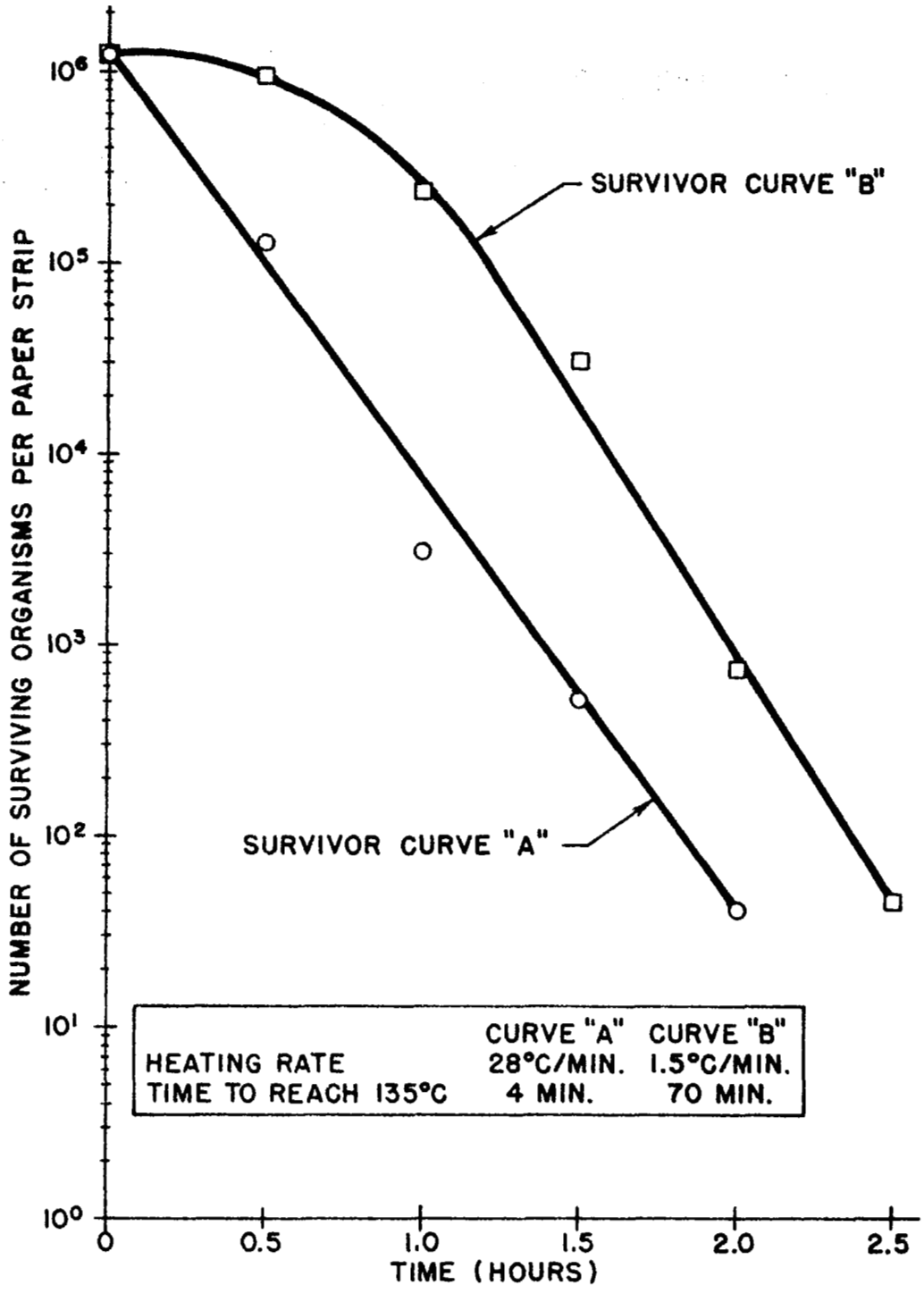

FIGURE 4. EFFECT OF THE WARM-UP RATE TO 135°C. ON DRY SPORES OF BACILLUS COAGULANS ON PAPER STRIPS WHEN EXPOSED TO DRY HEAT

TABLE 1

Thermal death times (F values) and D values (in hours) for dry heat sterilization at 257°F(125°C) of spores of Bacillus coagulans WH-9 dried on paper strips and sealed in ampoules under dry nitrogen and air[1]

	Air	Nitrogen
D values[2]	0.80	1.10
F values[3]	7.50	10.02
Experimental thermal death time	7.0	9.25

3 F values were calculated from the equation of Schmidt (1957).

2 D values were calculated from the equation of Stumbo (1948).

1 The sterility test medium for this organism was trypticase soy broth. Assays were made using Pederson's growth Agar (Rice and Pederson, 1954). Initial population was 8.6 x 10^6/strip. All incubation was at 37C.

TABLE 2

Preliminary observations on the number of dry spores of Bacillus subtilis var. niger on paper strips surviving 125°C in various gaseous environments.

Gaseous Environment	Type of Unit employed	Treatment Time (hr)	Number of Survivors (organisms/strip)
		NONTURBULENT	
		0 (Control)	3.3×10^7
AIR	aluminum block	3/4	1.5×10^7
		1	5×10^6
	Copper U-tube	3/4	1.4×10^7
		TURBULENT (FLOWING)*	
		1/2	$< 10^4$
AIR	Copper U-tubing	3/4	$\sim 1 \times 10^2$
		1	$< 10^2$
NITROGEN	Copper U-tubing	1	$\sim 2 \times 10^2$

* Flow rate of gas was 2.5 CFH

TABLE 3

Preliminary observations on the number of dry spores of Bacillus coagulans on paper strips surviving 125°C in various gaseous environment.

Gaseous Environment	Type of Unit employed	Treatment Time (hr)		Number of Survivors (organisms/strip)
		NONTURBULENT		
AIR	cylindrical aluminum block	0	(Control)	6×10^6
		1/2		2×10^6
		1-1/2		1.5×10^5
	copper U-tube	1/2	(vented to atmosphere)[a]	2.6×10^6
		1/2	(sealed)[b]	2.0×10^3
		1-1/2	(vented to atmosphere)	2.6×10^4
NITROGEN	copper U-tube	1/2	(sealed)	3.5×10^3
HELIUM	copper U-tube	1/2	(sealed)	4.9×10^3
		TURBULENT (FLOWING)		
AIR	copper U-tube	1/2		4.9×10^3(c)
	copper U-tube	1/2		4.1×10^3(d)
NITROGEN	copper U-tube	1/2		5.1×10^3(c)

(a) so that no pressure would develop
(b) a slight pressure could develop upon heating the sealed unit
(c) flow of gas was 2.5 CFM
(d) flow of gas was 25 CFM

TABLE 4

Recovery of microorganisms collected on membrane filters from various areas and the heat resistance of such samples

Sample Area	Sampling Period	No. of Samples	Average Number of Bacteria Per Filter Half[4]	Results of Heat Treatment of Organsims on Filter Halves		
				Temperature	Not Sterile	Sterile
Industrial Manufacturing area[1]	24 hr		51			
	48 hr		52	120C		1 hr
	5 days		113			
Microbiological Laboratory[2]	24 hr	44	10-100	120C		1 hr
Spore Room[3]	6 hr	3	450	135C		1 hr
Outdoor Air	24 hr	21		115C	up to 2 hr	
	24 hr	16	30-1000	120C		1 hr
	8 days	6		115C	-	4 hr

1 Dusty and dirty assembly area.
2 See table 5 for results of other sampling techniques.
3 Room in which spores of Bacillus subtilis var. niger were aerosolized due to laboratory operations. An average of 900-1000 spores of this organism were entrapped on each filter-half.
4 Aerobic mesophilic spore population as assayed after a heat shock of 65C for 30 min and plated on Plate Count Agar pour plates and incubated at 32C.

TABLE 5

Recovery of microorganisms collected in various manners from a microbiological laboratory and the heat resistance of such samples

Manner of Collecting sample	Sampling period	Volume of air sampled (cu.ft.)	Number of Samples	Average Number of Viable Organisms per Sample[1]	Results of heat treatment of samples		
					Temperature	Not Sterile	Sterile
					115°C	2 hrs	3 hrs
Filtration:					120	-	1
Membrane	1 days	605	44	84	135	15 min	-
Filter	3	1815	26	224	115	2 hrs	2 ½ hrs
	4	2420	18	80	115	3 hrs	4 hrs
	5		19	98	115	4 hrs	5 hrs
	6		9	212	115	4 hrs	-
	8		6	130	115	2 hrs	4 hrs
	9		9	196	115	4 hrs	-
	13	7865	19	294	115	2 hrs	3 hrs
	20		9	140	115	4 hrs	5 hrs
	30		12	162	115	4 hrs	5 hrs
Sedimentation:							
Glass	1 months		18	126			
Petri	2		15	98	Heat treatment of samples not completed		
Plates	3		16	103			
(15 cm diam)	4		9	295			
	6		9	251			
Microscope	1 months		10	22	115	3 hrs	4 hrs
slides	2		9	18			
(3 x 1 in)	3		4	40	115	4 hrs	-
	4		-	-			
	5		-	-	115	4 hrs	-

Glass jars (5.5 cm diam)	4 months		19	128			
	6		9	141	115	3 hrs	-
	8		4	-	115	5 hrs	-
Stainless Steel strips (2 x 0.25 in)	1 day		29	1-100	120C	-	1 hr
Liquid impinger (conc'd on membrane filter) (1.1 cu.ft/min)	½ hr		7	$10^1, 10^2$	Heat treatment of samples not completed.		
	1 hr		10				
	2 hr		12				
	3 hr		34				
Impactors	1 hr	60	15	166			
	2 hr	120	6	328			

1 Aerobic mesophilic spore population as assayed after a heat shock of 65C for 30 min. and plated on Plate Count Agar pour plates. Incubation temperature was 32C except for impactor samples which were total viable organisms at 32C incubation.

TABLE 6

Observation On The Levels Of Microbial Contamination On Items Selected From An Industrial Manufacturing Area

Level of Microorganisms Per Item	Number of Items Harboring		
	Bacteria Heat Shock[1]	Bacteria No Heat Shock	Molds
None	1	1	4
1-20	10	8	5
21-40	4	3	--
41-60	0	3	--
100	2	2	1

[1] 5 ml aliquots of rinse solution were heat shocked at 65°C for 30 min then plated.

TABLE 7

Observations On The Times Required To Sterilize Items Selected From An Industrial Manufacturing Area In Dry Heat at 135°C

Exposure Time	1.75 hr	3 hr	6 hr
Number of Items Not Sterilized	4	4	2*
Total Number of Items Treated	41	65	26

* These two items (plus 2 others) were cultured in baby food jars. All the other items (128) were cultured in screw cap tubes. None of 18 control jars containing sterile media showed contamination after two weeks incubation even when the lids were left ajar for as long as 48 hrs prior to incubation.

TABLE 8

Observations on the times (hours) required to sterilize samples of activated carbon with dry heat treatments

Material and Source	Type and Level of Contamination	Observation
1 - Will Corp. Brand (80 mesh) (Will Corporation Rochester, N. Y.) 0.01 g samples	natural microflora mesophilic aerobes 0.7-5x10^5/g mesophilic anaerobes 5x10^4/g thermophilic aerobes 2x10^4/g thermophilic anaerobes not detectable	Sterile after the following times at 135C: 1 hr in thioglycollate broth at 32C 3 hr in trypticase soy broth at 32C 6 hr in thioglycollate and trypticase soy broth at 55C
2 - Same as above except 0.1 g samples treated		Sterile after the following times at 135C: 3 hr in thioglycollate broth at 32C 5 hr in trypticase soy broth at 32C 6 hr in thioglycollate broth at 55C 12 hrs in trypticase soy broth at 55C
3 - Same as above #1 (sterilized) 0.01 g samples treated	with spores of B. subtilis var niger at a level of 1x10^7 per 0.01 g. Spores added from aqueous suspension then material dried in vacuum overnight.	Sterile after 1 hr at 160C in trypticase soy broth at 32C

TABLE 8 cont'd.

Material and Source	Type and Level of Contamination	Observation	
4 - Same as #3 above (sterilized) 0.01 g samples treated	with spores of B. subtilis var. niger at a level of 4.6×10^7 per 0.01 g. Spores added from an acetone suspension and dried overnight.	Sterile after: 8 hr at 135C 2 hr at 145C 0.5 hr at 160C	Cultured in trypticase soy broth at 32C
5 - Same as #3 above 0.01 g samples treated	with spores of B. subtilis var. niger at a level of 6×10^8 per 0.01 g. The organism was grown in the presence of sterile carbon black, harvested and dried with the carbon.	Sterile after: 8 hr at 135C 3 hr at 145C 0.75 hr at 160C	Cultured in trypticase soy broth at 32C.
Merck Brand #18351 (N. F. Powder) 0.01 g samples treated	natural microflora Levels not established	Sterile after the following times at 135C: 3 hr in thioglycollate broth 4 hr in trypticase soy broth at 32C incubation.	
Darco Brand G-60 (Atlas Chemical Industries, Inc. Wilmington, Mass.) 0.05 g samples treated	natural microflora mesophilic aerobes 8×10^4/g mesophilic anaerobes $3\text{-}30 \times 10^2$/g thermophilic aerobes 3×10^4/g thermophilic anaerobes $3\text{-}5 \times 10^4$/g	Sterile after: 3 hr at 120C in thioglycollate broth at 32C 1 hr at 132C in thioglycollate broth at 32C 1 hr at 120C in thioglycollate broth at 55C 0.25 hr at 135C in thioglycollate broth at 55C.	

* All samples incubated at least two weeks and subcultured at that time. Treatment was in 150 x 16 mm screw-cap test tubes in previously described dry heat units (Koesterer, 1962).
** Shortest time treated.

TABLE 9

Thermal death times and D values (in hours) at three temperatures for spores of Bacillus subtilis var. niger entrapped or added to various materials

Materials	Spores per test Sample	Times to Sterilize			D values*			
		115 239	125 257	135 275	115 239	125 257	135 275	°C °F
Paper strips	1.2×10^6	18	8	2.5	2.5	1.1	0.5	
Plaster of Paris	1.2×10^6	28	12	6	3.4	1.4	0.6	
Glue-base marble patching plaster	2.7×10^6	34	16	7	4.7	2.0	0.8	
Dental materials inlay investment B	2.1×10^6	44	22	10	6.1	2.8	1.1	
Bridge model material	1×10^6	48	22	8	6.4	3.0	0.9	

* The D values were calculated from the levels of spore contamination as found by assay of the solid materials on plate count agar. The samples were cylindrical pellets and the weight was approximately 0.33 g for all materials. Samples solidified around thermocouples indicated that all solids reached temperature within 10 min.

TABLE 10

Thermal death times and D values (in hours) at several temperatures for spores of *Bacillus coagulans* entrapped or added to various materials

MATERIALS	SPORES PER TEST SAMPLE	TIMES TO STERILIZE			D VALUES*			
		115 (239)	125 (257)	135 (275)	115 (239)	125 (257)	135 (275)	°C °F
Paper strips	$1.3x10^6$		7	3		0.94	0.44	
Plaster of Paris	$1.3x10^5$	24	14	6	4.2	2.1	0.90	
Glue-base marble patching plaster	$2.2x10^5$	26	18	8	4.2	2.6	1.0	
Dental materials: Inlay investment B	$2.0x10^5$	30	16	7	5.0	2.3	1.0	
Bridge model material	$1.1x10^5$	32	14	6	5.2	2.2	0.8	

* The D values were calculated from the levels of spore contamination as found by assay of the solid materials on plate count agar. The samples were cylindrical pellets and the wt was approximately 0.33 g for all materials. Samples solidified around thermocouples indicated that all solids reached temperature within 10 min. Assays for sterility of these materials were made in trypticase soy broth.

TABLE 11

Summary Of Investigation For Presence Of Contamination Within Various Electronic Components.

Type of Component	No. Contaminated[1] No. Tested
capacitor	2/20
diode	0/16
resistor	2/11
transistor	2/21
TOTAL	6/68

[1] Includes only those components with which there was definite growth on direct or diluted culture or where no doubt exists regarding both the evidence for the presence of contamination in the components and the absence of contamination in the isolator test system which could not be attributed to the component.

TABLE 12

Results Of Individual Tests For Presence Of Contamination Within Various Electronic Components

	Ref Line
Type of Component:	1
Component Number[1]:	2
Conclusion Regarding Presence of Microbial Contamination: (C = contaminated; NC = no contaminants found; Q = questionable presence of contaminants)	3
I. Results of Direct Culture of Component:	
1 - Surface sterility after decontamination of isolator system	4
2 - Direct culture of crushed component	5
3 - Dilution of direct culture (2)	6
II. Results of Control Tests Performed on Each Isolator System	
1 - Isolator	
a - biological indicators (spore strips)	
wall, front	7
wall, rear	8
wall, bottom	9
wall, top	10
glove, right	11
glove, left	12
exhaust filter	13

[1] Number assigned component as received from NASA. A confidential sheet has been supplied the Biosciences Office identifying the specific components as to manufacturer and specific type and series.

Code: + = growth of microorganisms
- = no growth of microorganisms evident
x = test not performed

	Ref Line
b - direct culture	
gloves, finger (dip into bottle of broth)	14
floor, work area (swab sample)	15
2 - Tools and Equipment	
prior to use - swab sample	16
prior to use - dip into bottle of broth	17
after use - swab sample	18
after use - dip into bottle of broth	19
3 - Incoming air (filtered through packed cotton)	
impinger on air line	20
bottle open to interior atmosphere during operation	21
III. Ability of Culture Medium to Support Growth	
1 - Control tubes exposed in isolator during sterilization cycle	22
Control bottles exposed in isolator during sterilization cycle	23
2 - Inoculation of above controls	24
3 - Inoculation of original direct culture vessel (I-2) with crushed component	25
4 - Inoculation of dilution of direct culture vessel (I-3) with crushed component	26
5 - Observation of any alteration of culture medium by crushed component.	27

RESULTS OF INDIVIDUAL TESTS

Line Number [1]	TRANSISTORS														
1															
2	37	38	39	40	41	42	43	44	45	46	47	48	49	50	81
3	NC	Q	NC	Q	NC	C Q	NC	Q	NC	Q	Q	NC	Q	NC	Q
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	x	-	x	+	x	-	x	-	x	-	x	-	-
6	-	-	x	-	x	-	x	-	x	-	x	-	x	-	-
7	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-
8	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
9	-	-	-	+	-	+	-	+	-	+	-	-	-	-	-
10	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-
11	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-
12	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-
13	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-
14	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-
19	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
25	-	+	x	+	x	x	x	+	x	+	x	+	x	+	-
26	+	+	x	+	x	+	x	+	x	+	x	+	x	+	+

[1] As per test description on pages 22 to 27.

RESULTS OF INDIVIDUAL TESTS

Line Number	TRANSISTORS														
1															
2	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
3	NC	NC	NC	NC	Q	NC	NC	NC	Q	Q	C Q	NC	NC	NC	NC
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
9	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
11	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-
19	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-
20	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
21	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
25	+	+	+	+	-	+	+	+	+	+	x	+	+	+	+
26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

RESULTS OF INDIVIDUAL TESTS

Line Number	TRANSISTORS					
1						
2	97	98	99	100	101	102
3	Q	Q	NC	Q	Q	NC
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	+	-
8	-	-	-	-	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	-	-	-	-	-	-
13	-	-	-	-	-	-
14	-	-	-	-	-	-
15	-	-	-	-	-	-
16	-	-	-	-	-	-
17	-	-	-	-	-	-
18	-	-	-	-	-	-
19	-	-	-	-	+	-
20	-	-	-	-	-	-
21	-	-	-	-	-	-
22	+	+	+	+	+	+
23	+	+	+	+	+	+
24	++	++	++	++	++	++
25	-	-	+	-	-	+
26	+	+	+	+	+	+

Totals:	36 items
C(but Q)	2
Q	14
NC	20

RESULTS OF INDIVIDUAL TESTS

Line Number	CAPACITORS																
1																	
2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
3	NC	NC	NC	NC	NC	Q	Q	NC	NC	Q	NC	NC	NC	NC	NC	C	C
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+
6	-	-	-	-	-	+	x	-	-	-	-	-	-	-	x	-	-
7	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-
8	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-
9	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
10	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	-
11	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-
12	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-
13	-	-	-	+	-	+	+	-	-	+	-	-	+	-	-	-	-
14	-	-	+	+	-	+	+	-	-	+	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
25	+	+	+	+	+	+	x	+	+	+	+	+	+	+	+	x	x
26	+	+	+	+	+	x	x	+	+	+	+	+	+	+	+	+	+

RESULTS OF INDIVIDUAL TESTS

Line Number	CAPACITORS														
1															
2	18	51	52	53	54	55	56	57	58	59	60	61	62	63	64
3	Q	Q	NC	Q	Q	Q	NC	Q	NC	NC	NC	Q	NC	Q	Q
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	+	-	x	-	x	-	x	-	-	-	x	-	x	-
6	-	x	-	x	-	x	-	x	-	-	-	x	-	x	-
7	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
12	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-
13	-	+	-	-	-	-	-	-	+	-	-	+	+	-	-
14	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
15	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-
22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
25	+	x	+	+	+	+	+	x	+	+	+	x	+	x	+
26	+	+	+	+	+	+	+	x	+	+	+	x	+	x	+

Totals: 32 items

C	2
Q	12
NC	18

RESULTS OF INDIVIDUAL TESTS

Line Number	DIODES														
1															
2	19	20	21	22	23	24	25	26	65	66	67	68	69	70	71
3	NC	NC	NC	NC	Q	Q	NC	NC	Q	NC	NC	Q	NC	NC	NC
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	x	-	x	-	x	-	x
7	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-
8	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-
9	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-
10	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-
11	-	-	+	-	+	-	-	-	-	+	-	+	-	-	-
12	-	-	-	-	+	-	-	-	-	+	-	+	-	+	-
13	+	-	-	-	+	-	+	-	-	+	-	+	-	-	-
14	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-
18	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	+	+	+	+	+	+	+	+	x	+	x	+	x		x

RESULTS OF INDIVIDUAL TESTS

Line Number					
1			DIODES		
2	72	73	74	75	76
3	NC	NC	NC	NC	NC
4	-	-	-	-	-
5	-	-	-	-	-
6	-	x	-	x	-
7	-	-	-	-	-
8	-	-	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-
11	-	-	+	-	-
12	-	-	-	-	-
13	-	-	-	-	-
14	-	-	-	-	-
15	-	-	-	-	-
16	-	-	-	-	-
17	-	-	-	-	-
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	-	-	-	-	-
22	+	+	+	+	+
23	+	+	+	+	+
24	++	++	++	++	++
25	+	+	+	+	+
26	+	x	+	x	+

Totals:	20 items
C	0
Q	4
NC	16

RESULTS OF INDIVIDUAL TESTS

Line Number	RESISTORS													
1														
2	27	28	29	30	31	32	33	34	35	36	77	78	79	80
3	NC	NC	NC	Q	NC	Q	C	NC	NC	NC	C	Q	NC	NC
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	+	-	-	-	+	+	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	+	-	-	-	-	-	+	-	+
8	-	-	-	-	-	+	-	-	-	-	-	+	-	+
9	-	-	-	-	-	+	-	-	-	-	-	+	-	+
10	-	-	-	-	-	-	-	-	-	-	-	+	-	+
11	-	-	-	-	-	+	-	-	-	-	-	+	-	+
12	-	-	-	-	-	+	-	-	-	-	-	+	-	-
13	-	-	-	-	-	-	-	-	-	-	-	+	-	+
14	-	-	-	-	-	-	+	+	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-	+
17	-	-	-	-	-	-	-	-	-	-	-	-	-	+
18	-	-	-	-	-	+	-	-	-	-	-	-	-	-
19	-	-	-	-	-	+	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	+	-	-	-	-	-	-	-	-	-	-
22	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	++	++	++	++	++	++	++	++	++	++	++	++	++	++
25	+	+	+	+	+	+	x	+	+	+	x	x	+	+
26	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Totals:	14 items
C	2
Q	3
NC	9

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